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Advances in Brief

Cytochrome P4501A1 and Glutathione *S*-Transferase (M1) Genetic Polymorphisms and Postmenopausal Breast Cancer Risk[1]

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Abstract

Polycyclic aromatic hydrocarbons, possible human breast carcinogens, are metabolized by cytochrome P4501A1 (*CYP1A1*) and glutathione *S*-transferase (*GSTM1*). A *CYP1A1* polymorphism (isoleucine to valine substitution in exon 7) or the null allele for *GSTM1* may affect the mutagenic potential of polycyclic aromatic hydrocarbons. We examined polymorphisms in *GSTM1* and *CYP1A1* in relation to breast cancer risk. Included were 216 postmenopausal Caucasian women with incident breast cancer and 282 community controls. DNA analyses suggested no increased breast cancer risk with the null *GSTM1* genotype [odds ratio (OR) = 1.10; CI, 0.73–1.64], although there was some indication that the null genotype was associated with risk among the youngest postmenopausal women (OR = 2.44; CI, 0.89–6.64). Slightly elevated risk was associated with the *CYP1A1* polymorphism (OR = 1.61; CI, 0.94–2.75) and was highest for those who smoked up to 29 pack-years (OR = 5.22; CI, 1.16–23.56). Statistical power to detect an effect may be limited by small numbers, and larger sample sizes would be required to corroborate these suggestive findings.

Introduction

PAHs[3] are known human carcinogens (1). Present in tobacco smoke and ubiquitous in urban environments, PAHs are lipophilic and stored in adipose tissue, including that of the breast (2). PAHs cause mammary tumors in rodents (3, 4) and are metabolized and activated by human mammary epithelial cells (5). PAHs have a high capacity for adduct formation in human breast cells (6, 7), and adduct levels in normal breast tissue have been found to be higher in women with breast cancer than in healthy controls (8). PAHs are metabolized to reactive intermediates by polymorphic cytochrome P4501A1 (*CYP1A1*) and detoxified by phase II enzymes, including glutathione S-transferase (*GSTM1*; Refs. 9 and 10). An amino acid exchange (isoleucine to valine) in exon 7 of *CYP1A1* has been linked to increased activity of the enzyme (11), and individuals who inherit the null allele for *GSTM1* are deficient for glutathione *S*-transferase (12). Some research has indicated that polymorphisms in *CYP1A1* and *GSTM1* may increase lung, bladder, and colon cancer risk (12–15). Because PAHs may be human breast carcinogens and *GSTM1* and *CYP1A1* are involved in carcinogen metabolism, we sought to determine if polymorphisms in these genes may be associated with increased risk of breast cancer, and if the association between genotypes and risk may be modified by cigarette smoking. Additionally, *GSTM1* deficiency has been associated with an earlier onset of lung cancer (12), prompting us to evaluate the association of age at postmenopausal breast cancer diagnosis with genetic polymorphisms in *GSTM1* and *CYP1A1*.

Materials and Methods

Study Population. This research used data collected in an earlier study of the epidemiology of postmenopausal breast cancer; the detailed methods have been reported previously (16). The protocol for the study was reviewed by the Institutional Review Board of the State University of New York at Buffalo, and informed consent was received from all participants. Postmenopausal, Caucasian women with incident, primary, histologically confirmed breast cancer (n = 439) were frequency matched by age and county of residence to controls (n = 494) randomly selected from the New York State Motor Vehicle lists (under age 65) and the Health Care Finance Administration rolls (65 years and older). Interview data included medical, reproductive, and life-style histories. All participants were asked to provide a blood specimen. Approximately 63% of postmenopausal women in the study consented to phlebotomy: 265 cases and 322 controls.

Laboratory Analysis. DNA was extracted from blood clots obtained following centrifugation and removal of serum, which were stored at −70°C. At the time of these analyses, the blood clots (1.0 ml) were polytroned and DNA was extracted using proteinase K and RNase digestion, followed by phenol extraction and ethanol precipitation, as described previously (17). The *CYP1A1* and *GSTM1* genetic polymorphisms were determined simultaneously. DNA was subjected to PCR in buffer [10 mM Tris-HCl (pH 8.3), 50 mM KCl, and 3 mM $MgCl_2$], 0.3 mM 2′deoxynucleoside-3′-triphosphates (Pharmacia, Piscataway, NJ), 2.5 units AmpliTaq DNA polymerase (Perkin-Elmer, Norwalk, CT), and primers specific for the *GSTM1* (20 pmol; 5′-CTGCCCTACTTGATTGATGGG and 5′-CTGGATTGTAGCAGATCATGC) and for the *CYP1A1* (20 pmol, 5′-GAAAGGCTGGGTCCACCCTCT (13) and 5′-CCAGGAAGAGAAAGACCTCCCAGCGGGCCA, *where the underlined base was substituted in order to introduce an* *Nco*I restriction site) in a total volume of 50 μl. As the primers for the *GSTM1* locus anneal to sites inside the coding region of the gene, the presence of the gene was determined by the presence of a band (273 bp), while the null genotype was determined by the lack of a band, using agarose gel electrophoresis (2.5%). The *CYP1A1* amplification served as an internal control. For the *CYP1A1* isoleucine to valine substitution (residue 462), a restriction enzyme site was entered into the primer that revealed the polymorphism with *Nco*I, as described previously (18). The amplified PCR product was subjected to restriction enzyme analysis with *Nco*I (New England Biolabs, Beverly, MA), according to the manufacturer's instructions. A simultaneous restriction enzyme digestion also was conducted with *Hin*fI (New England Biolabs, Beverly, MA), which cleaved the *GSTM1* fragment so that this band did not overlap the cleaved *CYP1A1* fragment. Analysis by gel electrophoresis (4.0% agarose; 3:1 NuSieve; FMC Bioproducts, Rockland, ME; Agarose, Gibco BRL, Gaithersburg, MD) revealed 232- and 31-bp fragments for wild-type alleles (isoleucine) or a single 263-bp fragment when the mutation (valine) was present. This assay has been validated by confirming a polymorphic inheritance pattern in eight family lines encompassing three generations (NIGMS Human Genetic Mutant Cell Repository, Coriell Institute, Camden, NJ). All assays were conducted and interpreted blinded to case-control status.

Statistical Analysis. Student's t tests were performed to assess mean differences in demographic, reproductive, and life-style factors by *GSTM1* and *CYP1A1* genotypes within case and control groups. To examine the associa-

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[3] The abbreviations used are: PAH, polycyclic aromatic hydrocarbon; *CYP1A1*, cytochrome P4501A1; *GSTM1*, glutathione *S*-transferase M1; OR, odds ratio(s); CI, confidence interval(s).

tions between *CYP1A1* and *GSTM1* genotypes and breast cancer risk, we calculated OR and 95% CI by unconditional logistic regression. The OR were adjusted for potential confounding factors, including age, education, body mass index, age at menarche, age at first pregnancy, age at menopause, and reported family history of breast cancer. To investigate the effect of both genotypes combined, because few women were homozygous for the *CYP1A1* mutant alleles, this category was collapsed with the *CYP1A1* heterozygotes. Unstratified associations were evaluated, as well as those within strata of age at diagnosis, according to quartile distributions of age among the controls. Possible modification of the associations between genetic polymorphisms and breast cancer risk were also evaluated by stratifying cases and controls into tertiles based on smoking histories. We examined risk associated with genotype among women who had never smoked and two categories of smokers divided at the median: lighter smokers (less than 29 pack-years) and heavier smokers (29 or more pack-years).

Results

Interpretable PCR assays varied for each genotypic analysis, depending on the quality of the DNA. Genetic data for *GSTM1* were available for 411 women, and for *CYP1A1*, 404 women. Distribution of *GSTM1* and *CYP1A1* genotypes and their relation to breast cancer risk among postmenopausal women are shown in Table 1. Approximately 50% of both cases and controls had the null *GSTM1* genotype, with no associated increased risk for breast cancer (OR = 1.10; CI, 0.73–1.64). For *CYP1A1* genotype, however, mutant alleles were more frequent in cases than in controls (20% *versus* 15%, respectively), although the difference was not statistically significant. With women who were homozygous for the wild-type allele (Ile;Ile) as the referent, there was an estimated relative risk of 1.53 (CI, 0.88–2.66) for women who were heterozygous for the valine substitution (Ile; Val). For those who were homozygous for the polymorphism (Val; Val), there was an OR of 2.85 (CI, 0.59–16.56). When heterozygotes and Val;Val homozygotes were combined, the OR for breast cancer among those with at least one valine allele was 1.61 (CI, 0.94–2.75). CI around these risk estimates included the null. We also evaluated the interactive effects of polymorphisms for both *GSTM1* and *CYP1A1* on risk. With women who had both wild-type *GSTM1* and *CYP1A1* as the referent category, there was no evidence of any interaction between the genotypes and breast cancer risk.

Because cases with the *GSTM1* null genotype were significantly younger than those with the wild-type alleles, associations between genotypes and breast cancer risk were assessed within quartiles of age categories (Table 2). Among the youngest postmenopausal women (less than 58), there was an elevated, although nonsignificant, increase in risk with the null genotype (OR = 2.41; CI, 0.90–6.48), but a null or inverse association for women in the older age categories. There was no clear effect of age on breast cancer risk associated with *CYP1A1* genotype.

Cigarette smoking did not modify the association between *GSTM1* genotype and breast cancer risk (Table 3). Associations between *CYP1A1* genotype and risk varied by smoking history, however. Although there was no increased risk among nonsmokers or heavier smokers (more than 28 pack-years), risk was elevated among lighter smokers with the isoleucine to valine substitution (OR = 5.22; CI, 1.16–23.56).

Table 1 Association of CYP1A1 and GSTM1 genetic polymorphisms with postmenopausal breast cancer risk (Western New York Breast Cancer Study, 1986–1991)

	Case *n* (%)	Control *n* (%)	OR (CI)[a]
GST WILDTYPE (+)	84 (48)	116 (50)	1.00
GST NULL (–)	93 (52)	117 (50)	1.10 (0.73–1.64)
Total	177	233	
CYP1A1 W/W	140 (80)	195 (85)	1.00
CYP1A1 W/M	32 (18)	31 (14)	1.53 (0.88–2.66)
CYP1A1 M/M	4 (2)	2 (1)	2.85 (0.49–16.56)
Total	176	228	
CYP1A1 W/W	140 (80)	195 (85)	1.00
CYP1A1 W/M[b]	36 (20)	33 (15)	1.61 (0.94–2.75)
Total	176	228	
CYP1A1 W/W GST+	57 (35)	91 (42)	1.00
CYP1A1 W/W GST–	71 (44)	95 (44)	1.17 (0.74–1.87)
CYP1A1 W/M[b] GST+	14 (9)	13 (6)	1.87 (0.80–4.36)
CYP1A1 W/M[b] GST–	19 (12)	18 (8)	1.74 (0.83–3.67)
Total	161	217	

[a] OR and 95% CI calculated with unconditional logistic regression, adjusted for age, education, age at menarche, age at first pregnancy, age at menopause, body mass index, and family history of breast cancer.

[b] Heterozygous mutant and homozygous mutant categories collapsed to form heterozygous mutant, where mutant is the isoleucine to valine substitution.

Table 2 Breast cancer risk associations with polymorphisms in GSTM1 and CYP1A1 in reference to wild-type genotypes, by age (Western New York Breast Cancer Study, 1986–1991)

Age (years)	Case *n* (%)	Control *n* (%)	Case *n* (%)	Control *n* (%)	OR (CI)[a]
	GSTM1 +[b]		*GSTM1* –[c]		
<58	14 (36)	34 (59)	25 (64)	24 (41)	2.44 (0.89–6.64)
58–63	20 (44)	31 (42)	25 (56)	43 (58)	0.91 (0.40–2.05)
64–69	27 (50)	27 (55)	27 (50)	22 (45)	1.11 (0.49–2.51)
>69	23 (59)	23 (45)	16 (41)	28 (55)	0.48 (0.18–1.32)
	CYP1A1 W/W[d]		*CYP1A1* W/M[e]		
<58	33 (83)	50 (89)	7 (18)	6 (11)	2.28 (0.53–9.75)
58–63	34 (76)	61 (82)	11 (24)	13 (18)	1.78 (0.61–5.20)
64–69	47 (87)	38 (84)	7 (13)	7 (16)	0.69 (0.18–2.64)
>69	27 (73)	45 (87)	10 (27)	7 (14)	2.15 (0.68–6.80)

[a] OR and 95% CI calculated by logistic regression, adjusted for age, education, age at menarche, age at first pregnancy, age at menopause, body mass index, and family history of breast cancer.

[b] *GSTM1* present.

[c] *GSTM1* null.

[d] Homozygous (Ile;Ile) for the *CYP1A1* wild-type alleles.

[e] Heterozygous (Ile;Val) and homozygous mutant (Val,Val) genotypes combined.

Discussion

In this study, we found the isoleucine to valine (Ile-Val) polymorphism in *CYP1A1* to be weakly, although nonsignificantly, associated with breast cancer risk. *GSTM1* genotype did not appear to affect risk in the overall data set, but the data were suggestive of an elevated risk among the youngest postmenopausal women. This study did not find that *GSTM1* and *CYP1A1* ~~poly~~morphisms had a multiplicative effect on breast cancer risk. Cigarette smoking did not affect the association between *GSTM1* and breast cancer risk but did appear to modify risk associated with the *CYP1A1* polymorphism.

To our knowledge, there has been only one previous study evaluating the association between the *CYP1A1* Ile-Val polymorphism and breast cancer risk (19). In that study, the polymorphism was not associated with risk, although the authors recognized a lack of statistical power in their data. In our data, 15% of the control population had the minor allele, consistent with prevalence reported in some other studies (12, 20), which resulted in limited power to detect an effect in this study also. Although it is possible that the *CYP1A1* polymorphism is not related to breast cancer risk, resulting in nonsignificant estimates of risk, the increasing OR with hetero- and homozygosity for the minor allele, and the borderline significance of the genotypes combined in our data, suggest that *CYP1A1* may be associated with breast cancer risk and should be evaluated in future studies.

The association between cigarette smoking, *CYP1A1* genotype, and breast cancer risk suggests that the polymorphism is associated with increased risk among lighter smokers only. It is possible that, among women with lower exposure to tobacco smoke, the Ile to Val polymorphism confers increased breast cancer risk, but that at higher levels of smoking, the supposed antiestrogenic effects of smoking (21)

TABLE 3 *Postmenopausal breast cancer risk associated with cigarette smoking by GSTM1 and CYP1A1 genotypes (Western New York Breast Cancer Study, 1986–1991)*

	Nonsmokers			Light smokers (<20 pack-years)			Heavy smokers (20 or more pack-years)		
Genotype	*Case n (%)*	*Control n (%)*	*OR (CI)*[a]	*Case n (%)*	*Control n (%)*	OR (CI)	*Case n (%)*	*Control n (%)*	OR (CI)
GST present (+)	37 (46)	54 (49)	1.00	20 (59)	28 (55)	1.00	17 (43)	22 (43)	1.00
GST null (−)	44 (54)	57 (51)	1.09 (0.61–1.98)	14 (41)	23 (45)	0.82 (0.31–2.16)	23 (58)	29 (57)	1.08 (0.43–2.72)
Total	*81 (100)*	111 (100)		34 (100)	51 (100)		40 (100)	51 (100)	
CYP1A1 W/W	66 (79)	91 (82)	1.00	24 (77)	50 (94)	1.00	36 (86)	40 (83)	1.00
CYP1A1 W/M[b]	18 (21)	20 (18)	1.30 (0.62–2.70)	7 (23)	3 (6)	5.22 (1.16–23.56)	6 (14)	8 (17)	0.86 (0.24–3.09)
Total	84 (100)	111 (100)		31 (100)	53 (100)		42 (100)	48 (100)	

[a] OR and 95% CI calculated by logistic regression, adjusted for age, education, age at menarche, age at first pregnancy, age at menopause, body mass index, and family history of breast cancer.

[b] Includes heterzygotes (Ile;Val) and homozygotes (Val;Val) for *CYP1A1* mutant allele

may overwhelm their carcinogenic potential among women with the minor allele. It is also possible that the finding of risk among lighter smokers is a function of the relatively small numbers of women in this category, resulting in unstable estimates of risk.

Our finding that the *GSTM1* null allele was not associated with breast cancer risk is consistent with the findings of other researchers (15).[4] Our data do suggest, however, that *GSTM1* may confer increased susceptibility to breast cancer at an earlier age. This is supported by research assessing *GSTM1* and lung cancer (12), where it was found that among individuals diagnosed at an earlier age, there was a preponderance of those with the null genotype. Epidemiological studies have found an association between earlier age of onset and family history of cancer for both lung and breast cancers (22, 23), implying that genetically susceptible individuals may develop cancer at an earlier age than those without the susceptibility. Particularly for cancers that may be related to exogenous or endogenous exposures resulting in electrophilic intermediates or free radicals, lack of glutathione *S*-transferase M1 may increase susceptibility, with greater DNA damage leading to carcinogenesis at an earlier age. Our study was restricted to postmenopausal women, and analysis of data from premenopausal women may further elucidate these issues.

Lack of statistical power to detect a true effect if it exists (type II error) is a common problem in molecular epidemiological studies, particularly those involving genotypes in which the polymorphism is rare. Power calculations indicate that, with a frequency of the *CYP1A1* minor allele at 15% in the control population, a sample size of 494 cases and an equal number of controls would be required to detect a relative risk of 1.61 at a significance of 0.05 with power of 0.80, *i.e.*, an 80% probability of detecting a statistically significant effect if it exists. Conversely, if a study sample is very large, test statistics for almost any variable may be "significant," yet reflect very minor differences between groups. Although it is possible that the weak or nonsignificant effects observed for the *CYP1A1* and *GSTM1* (among younger women) polymorphisms on risk are indicative of the lack of an association between genotypes and risk, the borderline significance and biological plausibility suggest that these areas of research need clarification. We would suggest that these findings be viewed as sentinels of research areas requiring further study, ideally with larger sample sizes. Continued examination of the role of *GSTM1* and *CYP1A1* polymorphisms in breast cancer risk, and their interaction with epidemiological variables, may further elucidate breast cancer etiology and the role that exogenous and endogenous substrates for enzyme products of these genes may play in breast carcinogenesis.

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